A DUAL EFFECT OF CARBON DIOXIDE ON INSECTS POISONED BY OXYGEN [1]

MARY HELEN M. GOLDSMITH AND HOWARD A. SCHNEIDERMAN

Botany Department, Yale University, New Haven, Conn., and Department of Biology, Western Reserve University, Cleveland, Ohio

The presence of small amounts of carbon dioxide during exposure to high pressure of oxygen accelerates the appearance of convulsions and death in oxygen poisoning of vertebrates (Hill, 1933; Shaw *et al.,* 1934). Several explanations have been offered to account for this phenomenon. For example, it was early proposed that during exposure to oxygen, carbon dioxide accumulates in the tissues, and that carbon dioxide is the effective agent in poisoning (Gesell, 1923). Other workers claimed that carbon dioxide contributes only secondarily to the lethal effect of oxygen itself (Shaw *et al.,* 1934), and the effect of carbon dioxide on oxygen poisoning of vertebrates is no doubt a complex one (Lambertsen *et al.,* 1953). Williams and Beecher (1944), in a study of oxygen poisoning in Drosophila, found that this sensitizing effect of carbon dioxide on oxygen poisoning was not restricted to vertebrates. They reported that the presence of small amounts of carbon dioxide increased the sensitivity of adult *Drosophila azteca* to 10 atmospheres (atms.) of oxygen. In view of these findings, we did not anticipate a curious result that we encountered while investigating oxygen poisoning in the parasitic wasp, *Mormoniella vitripennis:* carbon dioxide appeared to protect these wasps from oxygen poisoning (Goldsmith, 1955). This discovery, which found no parallel in the literature on oxygen poisoning, prompted a detailed study of the effects of carbon dioxide on oxygen poisoning of adult and developing insects.

Materials and Methods

1. *Experimental animals*

The chalcid wasp, *Marmoniella vitripennis* Walker, was used for these experiments. Procedures for rearing and handling this insect have already been described, along with detailed accounts of its life history and postembryonic development (Tiegs, 1922; Schneiderman and Horwitz. 1958; Goldsmith and Schneiderman, 1960). The present experiments utilized animals at three stages of development: (1) "pink stage" developing adults (24 hours after ecdysis from the final larval cuticle); (2) "black stage" developing adults (12 to 24 hours prior to adult emergence); and (3) adults (various ages, both males and females). Details of the method used to select insects of a uniform age and stage of development are given in a previous paper (Goldsmith and Schneiderman, 1960).

In most experiments with adult insects, 10 males or 10 females were placed in a one-dram shell vial, loosely plugged with cotton; a single vial was compressed

[1] This investigation was supported by a research grant from the National Heart Institute of the Public Health Service.

in each pressure chamber. The compression chambers were constructed of transparent polymethylmethacrylate (lucite), and the activity of adult wasps could be observed during exposure. Observations were begun when full pressure was reached and continued at ten-minute intervals until decompression.

Characteristically, normal wasps quickly right themselves if they fall or are knocked over. During the exposure to various gas mixtures, the times at which the first and last adults in a group became unable to right themselves, as well as the times at which the first and last adult ceased moving, were noted. In experiments in which the recovery of adults was to be studied, the chambers containing the experimental insects were decompressed 10 minutes after all the adults ceased moving. The activity of the wasps was observed immediately and then at intervals of 12 to 24 hours for several days. The reappearance of the righting behavior and the ability to walk were convenient measures of the extent of recovery. In observations during the recovery period, the wasps were knocked on their backs and the number which righted themselves as quickly as the control wasps was recorded. Most of the adults that could right themselves attempted to walk, but not all regained the agility of normal adults. Wasps that had recovered enough to run normally could, when tapped to the bottom of the glass vial, walk or run up the vertical side (30 mm.) within one minute. This last test was used as a convenient measure of full recovery. The activity and co-ordination of wasps which walked normally was not otherwise distinguishable from the control wasps which had not been exposed.

The responses of the sexes were similar, and results for males and females were averaged together. The data from each treatment were compared with those from other treatments in the same experiment. Each experiment was repeated at least once and usually several times.

2. *Compression and decompression*

Most experiments were carried out at 10 atms. of oxygen at 20° C., the same conditions used by Williams and Beecher (1944). The compression chambers enclosing the experimental wasps were equilibrated at the desired temperature for one-half to one hour prior to compression. In experiments with carbon dioxide, the desired pressure of carbon dioxide was superimposed on the atmosphere of air present in the compression chamber following equilibration at the experimental temperature. The amount of carbon dioxide added was monitored on a sensitive Bourdon-type gauge which had a capacity of one atmosphere (gauge pressure). After the addition of carbon dioxide, the compression chamber was sealed and the 1-atm. gauge was replaced by a gauge with a capacity of 20 atms. Following this, ten atms. of oxygen were added to the chamber within one minute. The procedure of adding the desired gases to the atmosphere of air already present in the tank insured that the insects were never subjected to oxygen tensions which were below normal. Throughout this paper all pressures are reported as gauge pressures.

Decompression was performed step-wise over a period of five minutes. Further details of these compression and decompression procedures have been given in an earlier paper (Goldsmith and Schneiderman, 1960).

Results

1. *Effects of carbon dioxide at normal oxygen tension*

Before appraising the influence of carbon dioxide on oxygen poisoning, it was necessary to assess the effects of carbon dioxide in the presence of a normal oxygen tension (0.2 atm.). The desired pressure of carbon dioxide was added to the atmosphere of air initially present in the compression chamber. Studies with nitrogen and helium have shown that small increases in ambient pressure, as would result from the added carbon dioxide, do not affect the insect's behavior. (Goldsmith, 1955).

As is well known carbon dioxide is commonly used as an anesthetic for insects. Ordinarily the movement and co-ordination of adult wasps were not conspicuously affected by one hour of exposure to 0.1 or 0.2 atm. of carbon dioxide. Occasionally, the wasps appeared less active in 0.2 atm. of carbon dioxide than in air, but they always maintained a standing posture, and they resumed normal activity as soon as they were returned to air. In 0.5 atm. of carbon dioxide, the wasps ceased moving after approximately 10 minutes.

The effectiveness of carbon dioxide was only slightly increased when 10 atms. of nitrogen were superimposed upon the carbon dioxide. Adults compressed with 10 atms. of nitrogen plus 0.2 atm. of carbon dioxide were only slightly less active than normal. However, when 10 atms. of nitrogen were superimposed upon 0.5 atm. of carbon dioxide, the insects ceased to move almost immediately. Since one atmosphere of air was present in all experiments, these results cannot be attributed to lack of oxygen. After four hours, the insects in 0.5 atm. of carbon dioxide and 10 atms. of nitrogen were decompressed. These wasps rapidly regained normal activity as did wasps decompressed after four hours in 0.5 atm. of carbon dioxide. From these results, it appears that in *Mormoniella* carbon dioxide at 0.1 atm. has no detectable effect, at 0.2 atm., a very slight effect, and at 0.5 atm., it is an effective anesthetic which has no detectable after-effects.

Table I

Duration of exposure to cause loss of spontaneous movements in adult Mormoniella exposed to various gas mixtures

Exp't No.	Number of wasps	Age of wasps (days)	Time at which first insect lost spontaneous movement to time at which entire population succumbed (minutes)		
			10 atms. O_2	10 atms. O_2 and 0.2 atm. CO_2	10 atms. O_2 and 0.5 atm. CO_2
26	200	0–1	70–80	55–65	not determined
33	95	0–1	40–50	35–45	0*
31	155	1–2	40–50	40–50	not determined
25	60	1–2	50–60	50–60	0
23	50	1–2	40–50	35–45	not determined
24	60	0–2	40–50	40–50	not determined
32	75	4–5	40–50	30–40	0

* Activity disappeared immediately.

2. *Effect of carbon dioxide on the onset of oxygen poisoning*

To appraise the effects of carbon dioxide on the onset of oxygen poisoning in adult wasps, insects in 10 atms. oxygen and 10 atms. oxygen plus 0.2 or 0.5 atm. carbon dioxide were observed at 10-minute intervals. Table I shows that complete loss of movement in all individuals consistently occurred within 10 minutes after the first wasp succumbed. In a few experiments (numbers 26 and 32), adults in oxygen plus 0.2 atm. of carbon dioxide appeared to lose spontaneous movement somewhat sooner than wasps in oxygen. This effect, however, was neither as marked nor as consistent (*cf.*, numbers 24, 25, 31) as that described by Williams

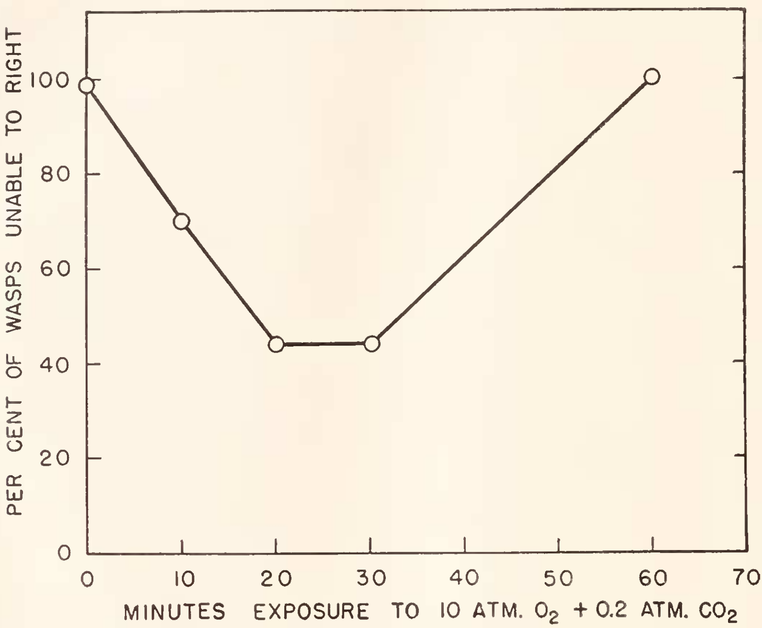

FIGURE 1. The loss of normal activity (as shown by loss of the righting reflex) during exposure to oxygen. Observations were made at 10-minute intervals on 80 adult wasps compressed in 0.2 atm. of carbon dioxide plus 10 atms. of oxygen. In this experiment, the activity of almost all the wasps was depressed initially, but there was a considerable return of activity before the paralytic symptoms of oxygen poisoning commenced (30 minutes).

and Beecher (1944) for 10- to 11-day-old *Drosophila*. They found that adults lost spontaneous activity almost twice as fast in oxygen mixed with 0.16 atm. carbon dioxide as in oxygen alone.

Invariably 0.5 atm. carbon dioxide plus 10 atms. oxygen immediately paralyzed adults, and they remained motionless for the duration of exposure. This was to be expected, since in control experiments 0.5 atm. carbon dioxide and 10 atms. nitrogen also had an immediate anesthetic effect.

In some experiments wasps in 0.2 atm. of carbon dioxide plus 10 atms. of oxygen ceased moving almost immediately; however, wasps that were affected in this way often recovered while still under compression.[2] This can be seen in the experiment in Figure 1. Immediately after compression, all but 1% of the wasps had lost their righting behavior. After 10 minutes about 30% had regained the ability to right themselves, and after 20 minutes more than 50% could right themselves. Thereafter anesthesia set in again, and after 60 minutes of compression none were able to right themselves.

This initial loss of activity and subsequent recovery was not invariable. In eight different experiments involving a total of 500 adults, 37% of the insects exhibited this initial paralysis. In three of these experiments none of the adults was initially affected, while in one experiment 99% became motionless immediately. It seems likely that this variability in the effect of 0.2 atm. carbon dioxide administered with 10 atms. oxygen can be attributed to variation in the sensitivity of different groups of wasps and to the fact that 0.2 atm. was approximately the threshold pressure of carbon dioxide for producing an immediate loss of co-ordination in 10 atms. oxygen. In the presence of 10 atms. oxygen, the initial depression of activity was never observed at pressures of carbon dioxide less than 0.2 atm., but at higher pressure (0.25, 0.5 atm.) of carbon dioxide, activity ceased almost immediately. Apparently in some cases, an insect's activity can be immediately, albeit temporarily, depressed by a mixture of carbon dioxide and oxygen at a carbon dioxide concentration which by itself is not anesthetic (0.2 atm.).

3. *Effect of the presence of carbon dioxide during oxygen poisoning on recovery*

Although the previous experiments revealed that under some conditions simultaneous exposure to carbon dioxide and oxygen resulted in almost immediate, but temporary, loss of co-ordination, the presence of carbon dioxide also prevented permanent injury by oxygen. Thus, wasps exposed to oxygen plus carbon dioxide showed striking recovery from the effects of oxygen poisoning.

Groups of wasps were kept compressed until 10 minutes after all visible movements had ceased. They were then decompressed, and their behavior observed for several days. Maximum recovery occurred within 48 hours. In a typical experiment (Fig. 2, Curve 1) at the time of maximum recovery, only 33% of the wasps that had been exposed to oxygen behaved normally. By contrast, most of those that had been exposed to oxygen and carbon dioxide recovered; in fact, all of those exposed to oxygen plus 0.2 or 0.5 atm. of carbon dioxide (Fig. 2, Curve 1) regained normal activity. Clearly, carbon dioxide has a marked protective effect.

The protective action of carbon dioxide was even more conspicuous when wasps were kept compressed for periods one and one-half times and twice that required to render them motionless (*i.e.*, up to 2½ hours). The results are recorded in Figure 2 (Curves 1.5 and 2). When the wasps were returned to air, none exposed to oxygen for 2½ hours survived; however, 27% of the wasps exposed for 2½ hours to oxygen and 0.2 atm. carbon dioxide, and 53% of the wasps exposed for the same period to oxygen plus 0.5 atm. carbon dioxide recovered their righting ability. The presence of small amounts of nitrogen, instead of the carbon dioxide,

[2] In these cases, the time given in Table I does not refer to this initial temporary paralysis, but to the time at which spontaneous movement ceased permanently under compression.

during the period of oxygen poisoning had no noticeable effect on the subsequent recovery of oxygen-poisoned insects.

4. *Effect of carbon dioxide during oxygen poisoning on the development of "pink" and "black stage" wasps*

Having demonstrated that carbon dixoide protects adult wasps from oxygen poisoning, our attention turned to developing wasps. Wasps in the "pink stage" of adult development were subjected to oxygen or oxygen and carbon dioxide for several hours, then decompressed, and their subsequent development recorded. An animal which attains the "black stage" has completed all the externally visible signs of development. During the period of four days which intervenes between the

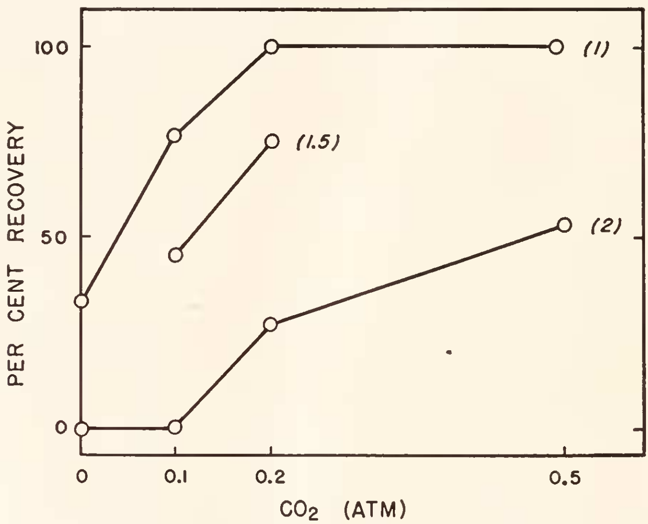

FIGURE 2. The effect of carbon dioxide on the recovery from oxygen poisoning (10 atms. at 20° C.). The ordinate gives the percentage of wasps exposed which had recovered the ability to right themselves 48 hours after decompression. Each curve shows the effect of a different duration of exposure. The basic exposure (1) continued for 10 minutes after spontaneous movements ceased, a total of 75 minutes. Other wasps were given 1.5 times and twice the basic exposure (Curves 1.5 and 2). Results obtained from 150 wasps.

"pink" and "black stages," external development is mainly concerned with epidermal pigmentation (Goldsmith and Schneiderman, 1960). Figure 3 reveals that all but about 5% of the wasps exposed to oxygen for 12 hours at the "pink stage" became black but only 13% of the exposed wasps were ever able to emerge fully; the rest remained completely or partially within the pupal cuticle. Although epidermal pigmentation was not affected, a system necessary for emergence was. These results agree with earlier experiments (Goldsmith and Schneiderman, 1960). By contrast, when carbon dioxide was present during exposure to oxygen, five times as many wasps (65%) emerged fully. Here again carbon dioxide exerted a protective action.

In another series of experiments, wasps were exposed to oxygen and carbon

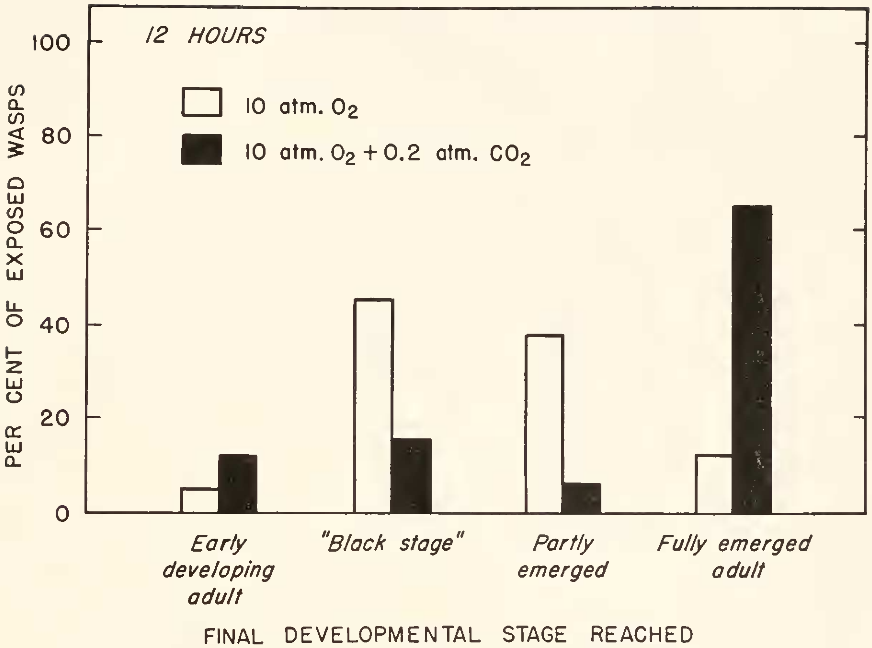

FIGURE 3. The final stage of development attained by "pink stage" developing adults after they were removed from 12 hours of 10 atms. of oxygen (open bars) or 12 hours of 10 atms. of oxygen plus 0.2 atm. of carbon dioxide (solid bars). Forty wasps were exposed to oxygen and 40 to oxygen and carbon dioxide.

dioxide during the "black stage" and again their ability to emerge was recorded. After 6 hours' exposure to oxygen, none of these wasps emerged completely and only 15% were active enough to free themselves partially from their pupal cuticle. The wasps that had been exposed to carbon dioxide plus oxygen were less severely affected; 5% emerged normally and 45% managed to emerge partially (Fig. 4).

5. *Effect of carbon dioxide on oxygen poisoning in Drosophila*

Experiments were also performed on a modest number of adults of *Drosophila melanogaster* (128 adults, 0 to 2 days old). When carbon dioxide was present during the exposure to oxygen, the flies lost their ability to make co-ordinated movements far sooner than in oxygen. Thus, it usually required 40 to 60 minutes of exposure to 10 atms. oxygen at 20° C. to abolish movement. By contrast, in seven separate compressions, all flies compressed with 0.2 atm. carbon dioxide plus 10 atms. oxygen ceased moving during the first 30 minutes of exposure. Such inactive flies did not recover while still compressed. Thus carbon dioxide administered with oxygen causes an initial loss of activity in *Drosophila* which is more marked than that observed in *Mormoniella*.

We may ask if the flies which became motionless with half an hour's or less exposure to carbon dioxide and 10 atms. oxygen were as severely affected as those subjected to oxygen until they were motionless. It is clear that they were

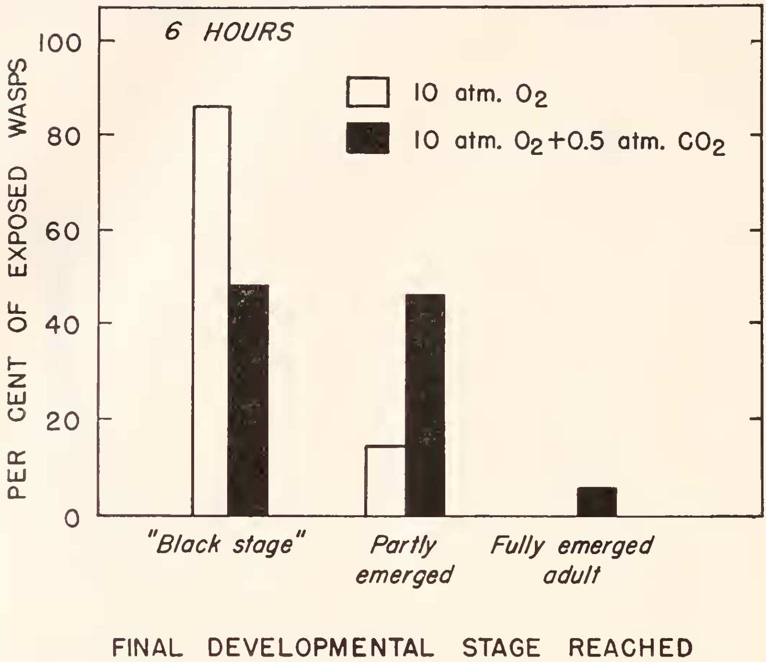

FIGURE 4. The emergence of "black stage" developing adults after they were removed from 6 hours of 10 atms. of oxygen (open bars) or 6 hours of 10 atms. of oxygen plus 0.5 atm. of carbon dioxide (solid bars). Forty wasps were used in each exposure.

not. When decompressed at the time they first ceased moving (*i.e.*, 10–30 minutes in oxygen and carbon dioxide, 40–60 minutes in oxygen), none of the former exhibited permanent injury; whereas, all but about 10% of the latter remained inactive (Table II).

Flies in oxygen and carbon dioxide were also subjected to the same length exposures that caused flies in oxygen without carbon dioxide to cease moving.

TABLE II

Survival of Drosophila melanogaster after oxygen poisoning

Exp't No.	Exposure (minutes)	10 atms. O_2		10 atms. O_2 and 0.2 atm. CO_2	
		Activity prior to removal	% Uninjured or recovered*	Activity prior to removal	% Uninjured or recovered*
21	10	Normal	100	None	100
40	30	Normal	100	None	100
21	40	None	0	Not determined	
43	50	None	20	None	50
40	60	None	10	None	40

* Per cent of adults uninjured is given for flies whose activity was normal on removal. Per cent of adults which recovered fully is given for flies who had lost all activity during exposure.

Under these conditions, it is evident that, as with *Mormoniella,* the presence of carbon dioxide during compression with oxygen promotes recovery of the flies. Thus while on the average only 10% recovered after an hour's exposure to oxygen, 40% recovered when 0.2 atm. of carbon dioxide was simultaneously present.

Discussion

From observations on adult *Mormoniella* and *Drosophila,* it seems clear that the presence of carbon dioxide during oxygen poisoning has at least two effects. First, it may hasten loss of activity without producing permanent toxic effects. Second, it promotes recovery and prevents permanent injury from oxygen poisoning. Oxygen poisoning in adult insects can be conveniently divided into two phases. In the first or reversible phase, the effects of poisoning are evident soon after compression, but if the insects are decompressed promptly there is no permanent damage. If decompression is delayed, the second or irreversible phase ensues; coordination becomes increasingly impaired, and the effects of poisoning persist indefinitely after decompression (*cf.* Williams and Beecher, 1944). Two interpretations of the effect of carbon dioxide in promoting the initial loss of activity are possible. Perhaps the presence of small amounts of carbon dioxide hastens the appearance of the reversible phase of oxygen poisoning or, alternatively, it may be that high pressures, particularly of oxygen, in some way potentiate carbon dioxide anesthesia. The present experiments do not allow us to choose between these two possibilities.

The protective action of carbon dioxide is exhibited with developing insects as well as adults. It has previously been shown that if the proper exposure is selected, wasps exposed to oxygen in the "pink stage" will develop to the "black stage" but fail to emerge. This inability to emerge results from failure of muscle development caused by oxygen poisoning (Goldsmith and Schneiderman, 1960). In the present study, many more wasps emerged after exposure to oxygen and carbon dioxide than after exposure to oxygen alone. Thus, "pink stage" wasps exposed to carbon dioxide and oxygen must have developed muscles.

In their study, Williams and Beecher (1944) concluded that the presence of carbon dioxide sensitized insects to oxygen poisoning. They examined the effects of carbon dioxide on 53 adults of *Drosophila azteca.* They found 10- to 11-day-old flies had lost all their activity when they were decompressed after 21 minutes in 10 atms. of oxygen at 20° C. The rate of poisoning (on the basis of the activity of the adults) was a linear function of the carbon dioxide tension (Williams and Beecher, 1944; Fig. 5). They reported that *Drosophila* in 0.16 atm. carbon dioxide plus 10 atms. oxygen ceased moving after 10 minutes. It is important to note that in this particular experiment they did not directly observe the flies during the compression period and did not look for recovery of the motionless flies after decompression. On the basis of our results, we suggest that the flies which Williams and Beecher exposed for brief periods to oxygen and carbon dioxide were not permanently injured, while at least some of the flies subjected to 20 minutes in oxygen alone probably suffered permanent damage. According to this view, there are no real differences between the results of the present experiment and those of Williams and Beecher. The conclusion of the present study is that although carbon dioxide may exert either a direct anesthetic effect in the presence of high pressures of oxygen or facilitate the appearance of the reversible phase of oxygen poisoning, the most striking effect of carbon dioxide is its effectiveness in preventing permanent damage from oxygen poisoning. It is provocative to speculate that the effectiveness of carbon dioxide in protecting insects from oxygen poisoning may be the result of its anesthetic properties, which could render the particular system that is sensitive to oxygen less susceptible to injury. Whether other narcotic agents similarly render protection from oxygen toxicity remains to be seen.

We wish to thank Professor C. M. Williams for his help in discussing this work, and in reading the manuscript.

Summary

1. The activity of adults of *Mormoniella vitripennis* and *Drosophila melanogaster* during and after compression in 10 atms. of oxygen plus small amounts of carbon dioxide (0.1, 0.2, and 0.5 atm.) was studied. Addition of carbon dioxide at pressures above 0.2 atm. to the atmosphere of air in the compression chamber anesthetized *Mormoniella* adults; on decompression all adults rapidly regained normal activity. The effects of carbon dioxide administered along with 10 atms. of nitrogen were similar except that with 0.5 atm. carbon dioxide, anesthesia occurred more rapidly.

2. Although in some experiments with adults of *Mormoniella* the presence of carbon dioxide during exposure to oxygen accelerated the onset of paralysis, carbon dioxide actually protected the adults from permanent injury caused by exposure to oxygen. After 2½ hours at 10 atms. about half of the adult wasps which had been in oxygen plus 0.5 atm. carbon dioxide completely recovered while none which had been in oxygen without carbon dioxide survived. The number of wasps which recovered increased as the amount of carbon dioxide present during oxygen exposure increased from 0.1 to 0.5 atm.

3. Although the presence of carbon dioxide did not totally prevent oxygen poisoning, permanent injury to "black stage" and "pink stage" developing adults as well as adult *Mormoniella* was significantly reduced.

4. In the presence of 10 atms. oxygen, adult *Drosophila* became motionless more quickly when 0.2 atm. of carbon dioxide was also present. These motionless flies recovered fully when decompressed. Furthermore, significantly fewer flies recovered following paralyzing exposures to oxygen without carbon dioxide.

LITERATURE CITED

Gesell, R., 1923. On chemical regulation of respiration. I. Regulation of respiration with special reference to the metabolism of the respiratory center and the coordination of the dual function of hemoglobin. *Amer. J. Physiol.*, **66**: 5–49.

Goldsmith, M. H. M. (née M. H. Martin), 1955. Studies on the mechanism of oxygen poisoning in insects and the effect of carbon dioxide on recovery. Thesis, Cornell University.

Goldsmith, M. H. M., and H. A. Schneiderman, 1960. The effects of oxygen poisoning on the post-embryonic development and behavior of a chalcid wasp. *Biol. Bull.*, **118**: 269–288.

Hill, L., 1933. The influence of carbon dioxide in production of oxygen poisoning. *Quart. J. Exp. Physiol.*, **23**: 49–50.

LAMBERTSEN, C. J., R. H. KOUGH, D. Y. COOPER, G. L. EMMEL, H. H. LOESCHKE AND C. F. SCHMIDT, 1953. Oxygen toxicity. Effects in man of oxygen inhalation at 1 and 3.5 atmospheres upon blood gas transport, cerebral circulation, and cerebral metabolism. *J. Appl. Physiol.*, **5**: 471–486.

SCHNEIDERMAN, H. A., AND J. HORWITZ, 1958. The induction and termination of facultative diapause in the chalcid wasps *Mormoniella vitripennis* (Walker) and *Tritneptis klugii* (Ratzeburg). *J. Exp. Biol.*, **35**: 520–551.

SHAW, L. A., A. R. BEHNKE AND A. C. MESSER, 1934. The role of carbon dioxide in producing the symptoms of oxygen poisoning. *Amer. J. Physiol.*, **108**: 652–661.

TIEGS, O. W., 1922. Researches on the insect metamorphosis. Part I: On the structure and post-embryonic development of a chalcid wasp *Nasonia*. *Trans. Roy. Soc. S. Aust.*, **46**: 319–527.

WILLIAMS, C. M., AND H. K. BEECHER, 1944. Sensitivity of *Drosophila* to poisoning by oxygen. *Amer. J. Physiol.*, **140**: 566–573.